PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	10.01.2001
Applicant's or agent's file reference: 126,447 PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL99/00519	30/09/1999	04/10/1998

Applicant
SHOENFELD, Yehuda et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 126,447 PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/IL99/00519	International filing date *(day/month/year)* 30/09/1999	Priority date *(day/month/year)* 04/10/1998
International Patent Classification (IPC) or national classification and IPC A61K38/17		
Applicant SHOENFELD, Yehuda et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/05/2000	10.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Deck, A Telephone No. +49 89 2399 8432

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL99/00519

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-9,14-22	as originally filed			
10-13	as received on	30/05/2000	with letter of	23/05/2000

Claims, No.:

1-6	as received on	30/05/2000	with letter of	23/05/2000
7-26	as received on	12/10/2000	with letter of	10/10/2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 14-26.

because:

   ☒ the said international application, or the said claims Nos. 14-26 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-26

	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations
see separate sheet

Concerning section III:

Claims 14 to 26 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Concerning section V:

The present application meets the requirements of Article 33 (2) and (3) PCT: the available prior art neither discloses nor suggests oral tolerance-inducing compositions for treatment of atherosclerosis, heart attack, angioplasty-restenosis or stroke comprising either LDL, Ox LDL, HSP 60/65, β_2GP-1 or functional derivatives thereof in combination with an oral carrier.

For the assessment of the present claims 14 to 26 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

U.S. Patent No. 5,348,945 discloses a method of combating mortality in a cell or tissue under stress. The method comprises contacting heat shock protein 70 (HSP70) to the cell or tissue in an amount effective to enhance the survival of that cell or tissue. The method may be employed in the combating of atherosclerosis, restenosis after angioplasty and nerve damage in human or animal subjects in need of such treatment. A pharmaceutical composition comprising a therapeutically effective amount of HSP70 in a pharmaceutically acceptable formulation is also disclosed.

Although HSP70 and HSP60 belong to a family of about 24 highly conserved heat shock proteins, they represent two entirely distinct characteristics. Their mechanism, for example, do not appear to act in concert in governing the protection from stressful stimuli.

HSP70 was initially patented because of its pronounced induction during heat exposure and other stressful insults such as ischemic preconditioning. Indeed the overexpression of HSP70 in transgenic animals is associated with protection from stressful hazards.

Therefore, U.S. Patent No. 5,348,945 does not teach or suggest the subject matter of the present invention.

Disclosure of the Invention

Thus, according to the present invention there is now provided an immunological and oral tolerance-inducing composition for prevention and/or treatment of atherosclerosis by the oral administration thereof, comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), $beta_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

In an preferred embodiment of the present invention there is provided an immunological and oral tolerance-inducing composition for prevention and/or treatment of a heart attack by the oral administration thereof, comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), $beta_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

In another preferred embodiment of the present invention there is provided an immunological and oral tolerance-inducing composition for prevention and/or treatment of angioplasty-restenosis by the oral administration thereof, comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

In a further preferred embodiment of the present invention there is provided an immunological and oral tolerance-inducing composition for prevention and/or treatment of stroke by the oral administration thereof, comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

In even further preferred embodiments of the present invention there is provided an immunological and oral tolerance-inducing composition wherein said active component is a modified low-density lipoprotein, or wherein said active component is oxidized low-density lipoprotein (Ox LDL), or wherein said active component is an active derivative of oxidized low-density lipoprotein (Ox LDL), or wherein said active component is heat shock protein 60/65 (HSP 60/65), or wherein said active component is beta$_2$-glycoprotein-1 (β_2GP-1).

The present invention provides an immunological and oral tolerance-inducing composition, wherein said active derivative is lysophosphatidyl choline (LPC).

The present invention also provides an immunological and oral tolerance-inducing composition, wherein said LDL is malondialdehyde LDL (MDA-LDL).

In another aspect of the present invention there is provided a method for prevention and/or treatment of atherosclerosis in a subject, comprising orally administering an immunological oral tolerance-inducing composition comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and

mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

In a preferred embodiment there is provided a method for prevention and/or treatment of a heart attack in a subject, comprising orally administering an immunological oral tolerance-inducing composition comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

In a further preferred embodiment there is provided a method for prevention and/or treatment of angioplasty-restenosis following angioplasty in a subject, comprising orally administering an immunological oral tolerance-inducing composition comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

In an even further preferred embodiment there is provided a method for prevention and/or treatment of stroke in a subject, comprising orally administering an immunological oral tolerance-inducing composition comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

In further embodiments of the present invention there is provided a method for prevention and/or treatment of atherosclerosis in a subject, wherein said active component is a modified low-density lipoprotein, or wherein said active component is oxidized low-density lipoprotein (Ox LDL), or wherein said active component is an active derivative of oxidized low-density lipoprotein (Ox LDL), or wherein said active component is heat shock protein 60/65 (HSP 60/65), or wherein said active component is beta$_2$-glycoprotein-1 (β_2GP-1).

The present invention further provides a method prevention and/or treatment of atherosclerosis in a subject, wherein said active derivative is lysophosphatidyl choline (LPC).

The present invention further provides a method prevention and/or treatment of atherosclerosis in a subject, wherein said LDL is malondialdehyde LDL (MDA-LDL).

The term "functional derivative" as used herein is intended to include labelled proteins, conjugated proteins, fused chimeric proteins and purified receptors in soluble form, as well as fragments, deletions, and conservative substitutions of said proteins.

The existence of an immune response against Ox LDL in atherosclerosis and the correlation between the reaction to Ox LDL and the severity of the disease, in combination with evidence that an active vaccine of Ox LDL in mice and rabbits can prevent the development of atherosclerosis has led the present inventors to conclude that the induction of immune tolerance by feeding Ox LDL to a human subject can result in the reduced rate of atherosclerosis progression. It should be mentioned that the mechanisms of inducing immune tolerance by mouth feeding are possibly mediated via a stimulation and production of cytokine TGFβ and the development of non-specific suppresser T-cells.

The oral tolerization of the present invention may extend to yield a bystandard suppression effect: namely - blocking other (non-antigen specific) autoimmune (anti-self) responses occurring in the vicinity of the atherosclerotic plaque and contributing to its progression.

It should be noted that the aim of the present invention is to induce tolerization or paralyze the immune response towards the HSP65, rather than to achieve mere elevation in the serum to assist protein unfolding.

Therefore, in one aspect the present invention combines oral tolerance, Ox LDL and atherosclerosis, which is a disease caused in part by immune factors. Ox LDL has been reported to induce an immune reaction in mice and rabbits (in contrast to inducing immune tolerance) of Ox LDL antigens and an improvement in the atherosclerosis condition. In these animal models Ox LDL has not been reported to have been experimented with mouth feeding and has never been suggested for oral tolerance.

WHAT IS CLAIMED IS:

1. - An immunological and oral tolerance-inducing composition for prevention and/or treatment of atherosclerosis by the oral administration thereof, comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

2. An immunological and oral tolerance-inducing composition for prevention and/or treatment of a heart attack by the oral administration thereof, comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

3. An immunological and oral tolerance-inducing composition for prevention and/or treatment of angioplasty-restenosis by the oral administration thereof, comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

4. An immunological and oral tolerance-inducing composition for prevention and/or treatment of stroke by the oral administration thereof, comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

5. An immunological and oral tolerance-inducing composition according to claim 1, wherein said active component is a modified low-density lipoprotein.

6. An immunological and oral tolerance-inducing composition according to claim 1, wherein said active component is oxidized low-density lipoprotein (Ox LDL).

7. An immunological and oral tolerance-inducing composition according to claim 1, wherein said active component is an active derivative of oxidized low-density lipoprotein (Ox LDL).

8. An immunological and oral tolerance-inducing composition according to claim 1, wherein said active component is heat shock protein 60/65 (HSP 60/65).

9. An immunological and oral tolerance-inducing composition according to claim 1, wherein said active component is an active derivative of HSP60/65.

10. An immunological and oral tolerance-inducing composition according to claim 1, wherein said active component is beta$_2$-glycoprotein-1 (β_2GP-1).

11. An immunological and oral tolerance-inducing composition according to claim 1, wherein said active component is an active derivative of β_2GP-1.

12. An immunological and oral tolerance-inducing composition according to claim 1, wherein said active component is an active derivative of LDL which active derivative is lysophosphatidyl choline (LPC).

13. An immunological and oral tolerance-inducing composition according to claim 1, wherein said LDL is malondialdehyde LDL (MDA-LDL).

14. A method for prevention and/or treatment of atherosclerosis in a subject, comprising orally administering an immunological and oral tolerance-inducing composition comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

15. A method for prevention and/or treatment of a heart attack in a subject, comprising orally administering an immunological and oral tolerance-inducing composition comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

16. A method for prevention and/or treatment of angioplasty-restenosis in a subject, comprising orally administering an immunological and oral tolerance-inducing composition comprising an active component selected from the

group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

17. A method for prevention and/or treatment of stroke in a subject, comprising orally administering an immunological and oral tolerance-inducing composition comprising an active component selected from the group consisting of modified low density lipoprotein, oxidized low density lipoprotein (Ox LDL), heat shock protein 60/65 (HSP 60/65), beta$_2$-glycoprotein-1 (β_2GP-1), functional derivatives thereof and mixtures thereof, in combination with a pharmaceutically acceptable carrier for oral administration.

18. A method according to claim 14, wherein said active component is a modified low-density lipoprotein.

19. A method according to claim 14, wherein said active component is oxidized low-density lipoprotein (Ox LDL).

20. A method according to claim 14, wherein said active component is an active derivative of oxidized low-density lipoprotein (Ox LDL).

21. A method according to claim 14, wherein said active component is heat shock protein 60/65 (HSP 60/65).

22. A method according to claim 14, wherein said active component is an active derivative of heat shock protein 60/65 (HSP 60/65).

23. A method according to claim 14, wherein said active component is beta$_2$-glycoprotein-1 (β_2GP-1).

24. A method according to claim 14, wherein said active component is an active derivative of beta$_2$-glycoprotein-1 (β_2GP-1).

25. A method according to claim 14, said active component is an active derivative of LDL which active derivative is lysophosphatidyl choline (LPC).

26. A method according to claim 14, wherein said LDL is malondialdehyde LDL (MDA-LDL).